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ACID PRODUCTION BY STREPTOCOCCUS VIRIDANS IN MEDIUMS OF DIFFERENT HYDROGEN-ION CONCENTRATION

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That the reaction of culture mediums is important is a mere reiteration of a statement found in every textbook on bacteriology. As to just how important it is we are only beginning to realize. Various reports have been made as to the optimum reaction for different organisms. It is quite probable, however, that much work will have to be done before it can be stated just what this is, for each known organism or group of organisms, with any degree of certainty.

In the literature we find numerous references to the initial reaction of given mediums used for the cultivation of the streptococcus. A bulletin from the Army Medical School suggests P_H 7.6 to P_H 7.8 for both the hemolytic and viridans varieties. Norton [1] says he uses agar with a P_H value of 8.1 as a basis for blood agar and obtains excellent results in growing both the streptococcus and pneumococcus. Avery and Cullen [2] used medium having an initial reaction of P_H 7.6 to P_H 7.8.

Since our work involves study of Strep. viridans, we determined to make an effort to find out whether a medium of one reaction was superior to that of another for growing this particular organism. In our experiments we assumed that the rate of acid production was a fair index of the rate of growth and vitality. It may be that the reaction which favors the most rapid growth will not produce an organism with the maximum degree of virulence. Such, indeed, seems to be the case with the diphtheria bacillus, as Bunker [3] found that the most rapid growth of the diphtheria bacillus occurs in a medium having an initial reaction between P_H 7.0 and P_H 7.5, while the greatest toxin production takes place in a medium having a reaction of P_H 7.8 to P_H 8.2.

We made four different tests, using cultures which varied in age from 14 up to 48 hours. Two different kinds of broth were used. The basis of both was beef extract (Liebig's) 0.3%; peptone (Difco brand), 1%; sodium chlorid,

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[1] Am. Jour. Public Health, 1919, 9, p. 190.
[2] Jour. Exper. Med., 1919, 29, p. 215.
[3] Jour. Bacteriol., 1919, 4, p. 379.

0.5%. This was made up and autoclaved at 20 pounds' pressure for 15 minutes. It was then divided into 2 parts. To one was added 1% glucose and to the other 5% ascites fluid and 0.2% glucose. Each of these was again divided into 5 parts and the parts given initial reactions of P_H 6.4, P_H 6.8, P_H 7.2, P_H 7.6 and P_H 8. We had then, practically, 10 different batches of broth. These were autoclaved at 15 pounds' pressure for 15 minutes, the reaction readjusted, filtered, tubed and autoclaved.

The ascites fluid used was first prepared by rendering it alkaline by adding 2.5% of a 40% sodium hydroxid solution and autoclaving it for 15 minutes at 15 pounds' pressure. It was then stored in the icebox until ready for use.[4]

In the experiment the simplified colorimetric method of determining the H-ion concentration as suggested by Barnett and Chapman[5] and somewhat elaborated by Norton[1] was used. Although this method is probably not suited for the most exact work, we believe it sufficiently accurate for adjusting the reactions of mediums. Checks made by the electrometric method have shown an error not exceeding 0.06.

Our results would seem to indicate that growth will take place in medium having a P_H value anywhere between 6.4 and 8.0. The index in this was that sufficient acid was produced in all the cultures in 24 hours to bring about the maximum color change in bromthymol blue. Further, all the tubes presented the typical appearance which indicates a growth, and subcultures all grew out in 24 hours.

There is an indication, however, that growth was distinctly retarded in medium which had a reaction more alkaline than P_H 7.6, and further, that the most rapid growth was obtained in medium which had an initial reaction of P_H 6.8.

The following is a detail of the procedure in one experiment, together with a table of the results. The others were all carried out in the same way, the only variation being the age of the culture used. This varied within the limits above given. As near as we could tell, the results were the same except that when using an older culture the acid production and the accompanying changes in the appearance of the medium will not take place quite as rapidly as it will with a younger one. The organism used was isolated from the root of a tooth, the apex of which had been the seat of infection.

One hundred c c of the 5% ascites broth, having an initial reaction of P_H 7.2, was inoculated and incubated 14 hours at 37 C. This was used for inoculating the test tube. The medium to be tested was given the different desired reactions, put in tubes—10 c c to a tube—and autoclaved at 15 pounds for 15 minutes. Five-tenths c c of the broth culture was introduced into each tube and all placed in the incubator at 37 C. At intervals one tube from each concentration was removed from the incubator and the reaction noted. The

[4] Report in press.

[5] Jour. Am. Med. Assn., 1918, 70, p. 1062.

reaction immediately after inoculating was also noted to serve as a control. A set of uninoculated tubes was also incubated and the reactions noted after 25 hours. The reason for using separate tubes instead of having the medium in bulk and taking out the amount to be tested each time was to avoid any chance of contamination. Numerous checks have served to show that the reaction of one tube is practically the reaction of all the tubes of that concentration and composition at that time. This, of course, is as it should be since each tube contained the same amount of medium and each was inoculated with an equal amount of the culture. We made no attempt to determine the P_H value after it became more acid than P_H 6.3 except to note whether it would bring about the maximum color change in bromthymol blue. This would indicate a higher degree of acidity than P_H 6.3. In this experiment we were concerned only with the rate of acid production and were making no effort to determine the final H-ion concentration.

Ascites broth having an initial reaction of P_H 6.8 reached this end point after 6 hours' incubation while glucose broth of the same concentration reached the same point after 7 hours' incubation. The broth having an initial reaction of P_H 6.4, of course, closely approximated this. But since a comparative slight addition of acid would be sufficient to bring this medium to the end point one would expect that if an equal degree of acid had been produced in each tube, those of a P_H value of 6.4 would reach the end point first. With the ascites broth this was not the case. It was with the glucose broth, but there is not sufficient difference to justify such an assumption. When comparing the broth of concentration P_H 6.8 with those of a more alkaline reaction, it will be noticed that there is a difference of from 3-6 hours in the time required to reach the end point. In the case of ascites broth, P_H 8, there was no appreciable acid production until after 12 hours' incubation.

The gross appearance of the tubes corresponded to the rise in P_H value, that is, as the tubes became more acid they also became more turbid.

Although nearly all textbooks, when describing cultural characteristics of various organisms, speak of the turbidity or cloudiness and the formation of precipitates in liquid mediums, the reason for such reactions and just what they indicate is not made clear. Although the student is told that such changes in the appearance of mediums indicate that bacteria are present, he is not always told why their presence produces such a change.

Kendall[6] dismisses the matter by saying that visible changes in the appearance of broth cultures incidental to the development of bacteria are not of great importance, and mentions the fact that they consist essentially of turbidity, sediment and occasionally a ring or pellicle.

It is a significant fact, however, that all bacteria do alter the physical appearance of fluid mediums, and that this fact is always referred to in some way. So important is it that in the fabrication of

[6] Bacteriology, General, Pathological and Intestinal, 1916.

fluid mediums, clearness is insisted on whenever possible in order that these changes may be the more readily detected.

Like all other living organisms, the metabolic processes of bacteria may be divided into two phases, anabolism and catabolism. In the case of bacteria the catabolic phase predominates.

Any alteration in the composition or appearance of mediums due to anabolic processes are so slight that they can be disregarded. During the first few hours after inoculation the anabolic phase may go on quite rapidly and a slight cloudiness may result in fluid mediums, due chiefly to the rapid increase in the number of bacterial cells.

This phase is rapidly succeeded by the catabolic one and then the greater changes, due to the breaking down of the various constituents of the mediums, take place, and with it the accompanying changes in the physical appearance are noted.

Without going into great detail, since the details have been ably set forth by many, these catabolic changes are divided into two main groups: those that have to do with the breaking down of proteins and those that are concerned in the splitting up of sugars.

All bacteria do not possess the power to do both, nor can all bacteria split up both proteids and sugars to the same extent. The resultant end products produced by different types of organisms have been studied and used as an aid in identification.

One of the principal end products resulting from the activity of both proteolytic and sugar splitting enzymes is an acid in one form or another. Not only will this acid alter the reaction of the medium, but it will often precipitate substances that were held in solution; for example, phosphates. These precipitates will be the principal factor in the change from a clear to a cloudy medium. Some of the end products are only slightly soluble, and as they are formed they appear as precipitates or suspensions.

All of these changes are important and indicate that an organism is present. The changes are not only due to the presence of myriads of bacterial cells, but also to alterations in the composition of the medium resulting from bacterial metabolism. Eventually the organism may die, due either to the exhaustion of the available food supply or to the liberation of so much in the way of decomposition products that the environment is no longer favorable to its existence.

An analogous change is often noted when adjusting the reaction of mediums. If the medium is too alkaline it becomes necessary to add acid, usually normal hydrochloric, to bring it to the proper point. The

addition of such an acid always throws down a precipitate, either at once or on heating. This precipitate can, of course, be filtered out. It is reasonable to suppose that acid produced as the result of bacterial metabolism would also bring about such a change.

It is a significant fact that in the experiment herein described, the typical indications of a vigorous growth, such as cloudiness and the formation of a precipitate, only appeared as the degree of acidity approached P_H 6.3. Not until they reached this point would a mere inspection of the tubes reveal the presence of a culture. Control tubes of uninoculated medium which were incubated with these cultures did not show any such changes.

TABLE 1

RESULTS OF EXPERIMENTS

Original Reaction before Final Tubing and Autoclaving	Reaction just before Inoculating	Reaction just after Inoculating	Reaction after 3 Hours' Incubation	Reaction after 6 Hours' Incubation	Reaction after 7 Hours' Incubation	Reaction after 8 Hours' Incubation	Reaction after 9 Hours' Incubation	Reaction after 12 Hours' Incubation	Reaction after 25 Hours' Incubation	Reaction of Uninoculated Tubes after 25 Hours' Incubation
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
5% ascites, 0.2% glucose, P_H 6.4	6.4	6.4	6.4	6.3	6.3—	...	...	...	...	6.4
5% ascites, 0.2% glucose, P_H 6.8	6.8	6.7	6.6	6.3—	...	...	...	...	...	6.6
5% ascites, 0.2% glucose, P_H 7.2	7.3	7.3	7.3	6.7	6.6	6.4	6.4	6.3—	...	7.3
5% ascites, 0.2% glucose. P_H 7.6	7.6	7.6	7.4	7.2	6.9	6.6	6.3	6.3—	...	7.6
5% ascites, 0.2% glucose, P_H 8.0	8.0	8.0	8.0	7.8	7.8	7.8	7.8	7.8	6.3—	8.0
1% glucose..... P_H 6.4	6.4	6.4	6.4	6.3—	...	...	...	...	...	6.4
1% glucose..... P_H 6.8	6.7	6.6	6.7	6.3	6.3—	...	...	...	...	6.6
1% glucose..... P_H 7.2	7.0	7.0	6.6	6.4	6.3	6.3	6.3—	...	...	7.0
1% glucose..... P_H 7.6	7.4	7.4	7.4	6.8	6.8	6.4	6.3	6.3—	...	7.4
1% glucose..... P_H 8.0	7.4	7.4	7.4	6.8	6.9	6.6	6.4	6.3—	...	7.4

P_H 6.3— is used to indicate the maximum color change in bromthymol blue.

The chief source of acid production in our medium is the glucose content. It is shown by Avery and Cullen [2] that the amount of glucose, at least in concentrations between 0.5% and 1.5%, does not influence the final H-ion concentration or to any appreciable extent the rate of acid production. According to their experiment, too, very little acid is produced in a plain or sugar-free broth. The slight increase in acidity which they do show might easily be due to the final sterilization and in no way to the action of bacteria. They do not speak of any control which covers this point.

Our own experiments show a somewhat greater amount of acid produced in plain broth. We inoculated several series of 10 tubes each with a Streptococcus viridans and incubated them for 6 days. The P_H value of the broth just before inoculating was between 7.1 and 7.3. At the end of this time the reaction in all tubes was P_H 6.6. Control tubes of uninoculated medium incubated for the same length of time showed no change in reaction. This broth was made from beef extract and was reasonably free of sugar. It was not made absolutely so by fermenting with B. coli, however.

CONCLUSIONS

While there may not be a great deal to choose between P_H 6.4 and P_H 7.6, a broth given an initial reaction of P_H 6.8 will favor a more rapid growth, while a broth having a reaction more alkaline than P_H 7.6 will distinctly retard growth. Between the 5% ascites, 0.2% glucose broth and the 1% glucose broth there appears to be no appreciable difference when growing a pure culture.